MACROCYCLIC SPERMIDINE AND SPERMINE ALKALOIDS

M. M. BADAWI[a], K. BERNAUER[c], P. VAN DEN BROEK[a], D. GRÖGER[b], A. GUGGISBERG[a], S. JOHNE[b], I. KOMPIŠ[a], F. SCHNEIDER[c], H.-J. VEITH[a], M. HESSE[a] and H. SCHMID[a]

(a) *Organisch-chemisches Institut der Universität Zürich, Switzerland*
(b) *Institut für Biochemie der Pflanzen, Deutsche Akademie der Wissenschaften, Halle/Saale, DDR*
(c) *Chemische Forschungsabteilung der F. Hoffmann–La Roche & Co. AG, Basel, Switzerland*

ABSTRACT

In this short review of spermine and spermidine plant alkaloids, which are characterized by the presence of a macrocyclic lactam ring, special consideration has been given to the alkaloids from Oncinotis species and to the spermine alkaloid, chaenorrhine.

1. INTRODUCTION

The bases putrescine (**1**), spermidine (**2**), and spermine (**3**), are widely distributed in the animal kingdom and in micro-organisms[1,2], as is also putrescine in plants[2,3]. Quite recently, however, spermidine and spermine have been found, admittedly only in small amounts, in more highly cultivated plants such as cereals, cabbage, spinach, artichokes, etc., see[2,3] and the literature given there.

Sym.-homospermidine (**4**) has also been found quite recently in the leaves of *Santalum album* L.[4].

The biosyntheses of spermidine and spermine were investigated mainly in micro-organisms[2] and in rat liver[5]. Putrescine in plants is derived from ornithine or arginine[6]. The main interest for us lies in the plant putrescine derivatives containing an amide group, formed by combination of the base with *p*-hydroxy cinnamic acid or derivatives of it. Examples are paucine from Leguminose *Penthaclethra macrophylla* Benth.[7], the putrescine amides from *Kniphofia flavovirens*, *K. foliosa* and *K. tuckii* (Liliaceae)[8], subaphylline from *Salsola subaphylla* (Chaenopodiaceae)[9] and *Citrus* spp. (Rutaceae)[10].

Sym.-homospermidine (**4**) occurs as part of the alkaloids solapalmitine (**5**) and solapalmitenine (**6**) in *Solanum tripartitum* Dunal (Solanaceae)[11]. Both of these alkaloids possess tumour-inhibitory properties. The spermidine and spermine alkaloids, so far known, differ from the above mentioned amides by the presence of a macrocyclic lactam ring, formed by combination of the base with long-chain fatty acids or cinnamic acid and derivatives.

$H_2N{-}(CH_2)_4{-}NH_2$
1, Putrescine

$H_2N{-}(CH_2)_3{-}NH{-}(CH_2)_4{-}NH_2$
2, Spermidine

$H_2N{-}(CH_2)_3{-}NH{-}(CH_2)_4{-}NH{-}(CH_2)_3{-}NH_2$
3, Spermine

$H_2N{-}(CH_2)_4{-}NH{-}(CH_2)_4{-}NH_2$
4, sym.-Homospermidine

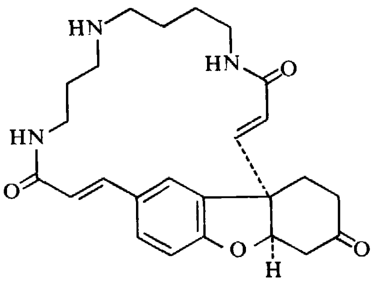
$(CH_3)_2N{-}(CH_2)_4$
N—R
$(CH_3)_2N{-}(CH_2)_4$

5, R = $-CO-CH\overset{tr.}{=}CH-(CH_2)_{12}-CH_3$, Solapalmitenine
6, R = $-CO-(CH_2)_{14}-CH_3$, Solapalmitine

The first example of this new alkaloid group was the spermidine alkaloid lunarine (7), isolated by E. Hairs[12] in the year 1908 from *Lunaria biennis* Moench (Cruciferaceae). Its correct structural formula **7** was first established in 1965 by G. A. Sim and J. A. D. Jeffreyes by a detailed x-ray structural analysis of lunarine-hydrobromide monohydrate and the corresponding hydroiodide monohydrate[13]. The next alkaloid of this group to be isolated was palustrine from marsh horse tail *Equisetum palustre* L. (Equisetaceae) by Glet *et al.*[14]. C. H. Eugster was the first to show that potassium hydroxide fusion of the alkaloid gave the basic amine, spermidine, as well as some lower amines[15]. Alkali fusion has become of general importance for this group of alkaloids as spermine (**3**) and spermidine (**2**) can now be isolated relatively easily in the form of their *N*-acetyl derivatives and recognized with certainty by their characteristic fragmentation pattern in the mass spectrometer[16].

Somewhat later Wiesner and his group isolated pithecolobine from *Samanea saman* Merr. (=*Pithecolobium saman* Benth.) (Leguminosae)[17,18]. Like the orginally postulated formula for lunarine[19], the suggested structural formula for palustrine, which still did not contain a tetrahydropyridine ring, and that for pithecolobine with a 27-membered lactam ring, had to be revised.

The spermidine and spermine alkaloids known to this date are now



7, Lunarine

discussed. Alkaloids of this group which have been recognized still more recently are the outcome of investigations carried out during the last few years (since 1968).

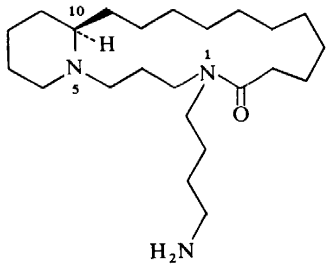
2. SPERMIDINE ALKALOIDS

2.1. Oncinotine

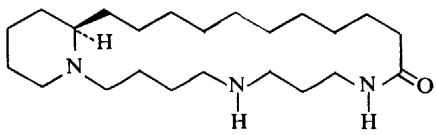
In the course of our studies on the indole alkaloids from apocynaceae we also investigated the bark from the stems and roots of *Oncinotis nitida* Benth. (indigenous to West Africa) and isolated, instead of the expected indole alkaloids, several spermidine alkaloids. The principal one was the non-crystalline oncinotine (**8**, $C_{23}H_{45}N_3O$) with its seventeen membered lactam ring, accompanied by, among others, its isomer isooncinotine (**9**, $C_{23}H_{45}N_3O$)[21].

By infra-red and mass spectrometric investigations of oncinotine (**8**), its mono-acetate, its lithium aluminium hydride reduction product and the acetyl derivative therefrom, it could be concluded that the alkaloid possessed two basic nitrogen atoms and a lactam group. The compound **8** shows in its n.m.r. spectrum no absorption for vinyl protons. Its lithium aluminium hydride reduction product shows at 195 nm only slight end absorption: C=C bonds are also absent.

The region from 3.0 to 0.7 p.p.m. in the 100 MHz-n.m.r.-spectrum is so complex that it was impossible to decide on the presence or absence of a (C)—CH_3 group. The classical Kuhn–Roth–C-methyl analysis had therefore to be resorted to in order to demonstrate its absence.



8, Oncinotine

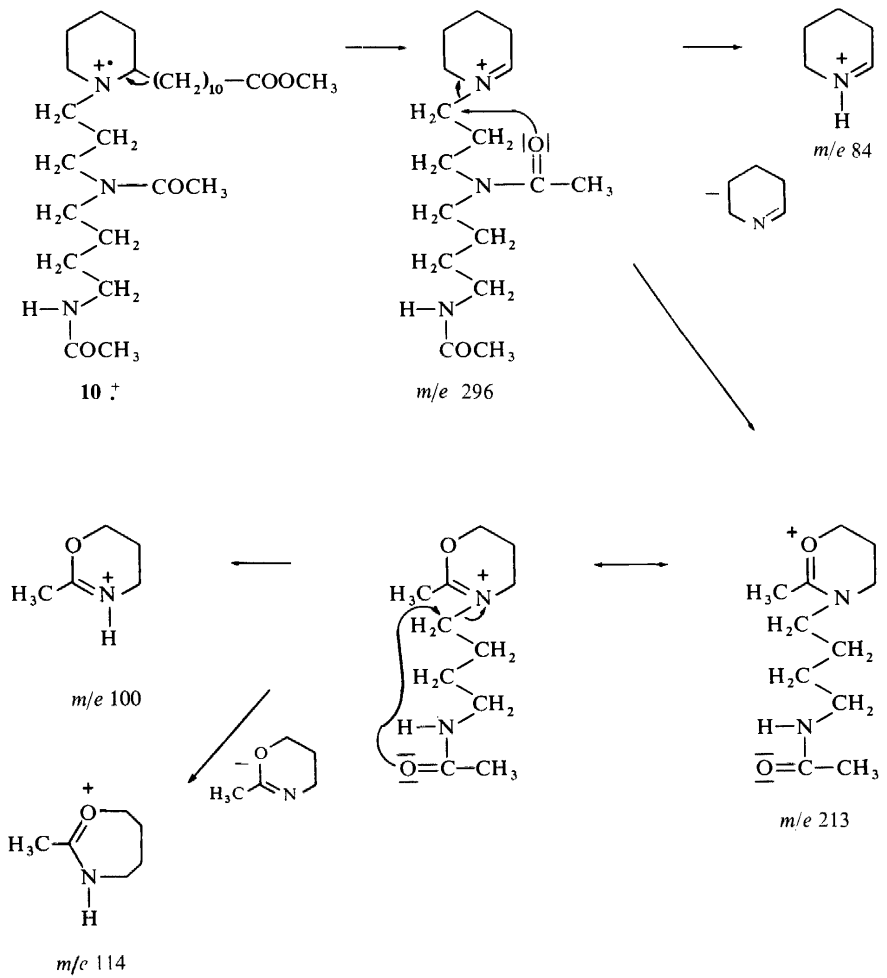


9, Isooncinotine

The following degradation was particularly important for the determination of the structure of oncinotine: Acid-catalysed hydrolysis of the lactam ring followed by esterification and *N*-acetylation of the resulting amino acid gave the ester acetate **10**, which in the mass spectrometer underwent the following characteristic fragmentation reactions. (*Scheme 1*)[23].

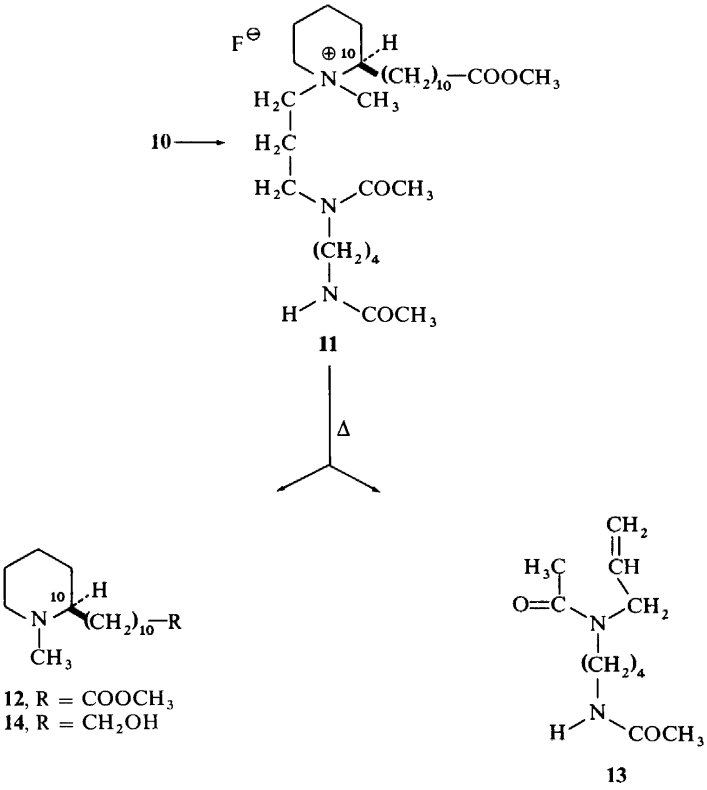
Conversion of **10** into the methofluoride **11**, followed by pyrolysis gave by Hofmann degradation the two bases **12** and **13** (*Scheme 2*) cf.[22]. In the mass spectrum the degradation product **13** breaks down according to *Scheme 3*[16]. The observation that the molecular ion $\mathbf{13}^+$ does not split off a C_3H_5 residue indicates that in oncinotine (**8**) and its derivatives, the 1,3-diaminopropane part and not the putrescine part of spermidine is incorporated into the lactam ring. It follows that **8** is the formula for oncinotine.

Scheme 1.



The two degradation products, **12** (in racemic form) and **13**, were synthesized. The synthetic products possessed, apart from rotation, the same physical properties as the degradation products of oncinotine. The reduction product **14** from **12** showed the following c.d.: $[\theta]_{230} = 0$, $[\theta]_{197} = -4500$. In comparison, *R*(−)-*N*-methyl coniine (**15**) of known absolute configuration[24] gave the following values: $[\theta]_{240} = 0$, $[\theta]_{200} = -3100$. It follows that oncinotine (**8**) and its derivatives possess the *R*-configuration at centre 10.

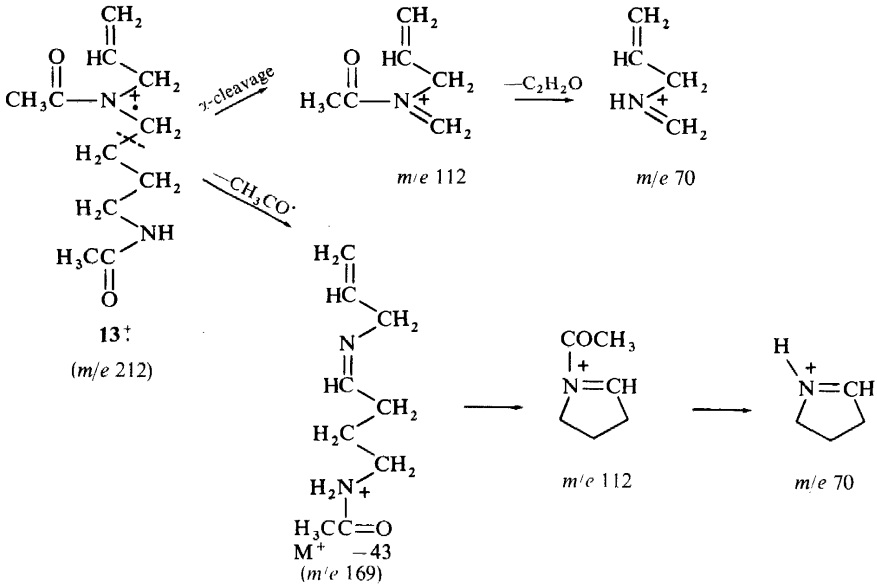
Scheme 2.



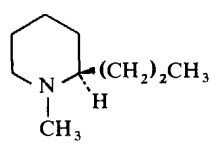
In the alkaloid iso-oncinotine (**9**) spermidine (**2**) is built in the reverse way as compared with oncinotine (**8**). In the meantime, a new C_{23}-alkaloid, neo-oncinotine, was found in *O. nitida*; this alkaloid shows the same type of spermidine incorporation as iso-oncinotine (**9**) and has an eighteen membered lactam ring[25].

Rac. oncinotine [(±)-**8**] has been synthesized starting from sebacic acid methyl ester and α-picolyl lithium and involving intermediate **16** according to *Scheme 4*. The yields at the various stages vary within the normal limits.

Scheme 3.

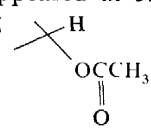


The tri-hydrochloride **17** is crystalline: its cyclization to the true alkaloid (±)-**8** has so far been realized only in poor yields. Alternative syntheses are now being worked out.



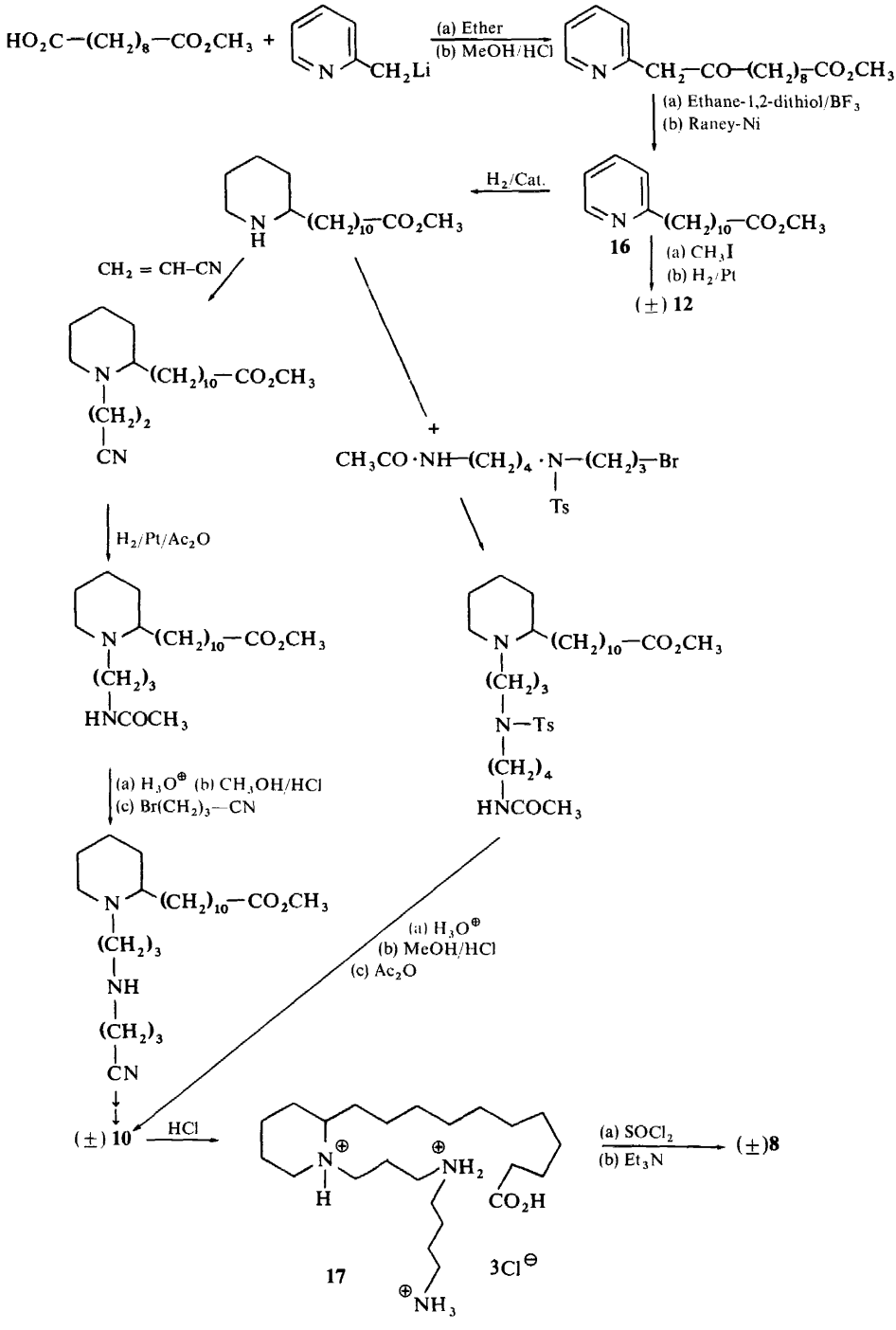
15, R(−)-*N*-Methyl-coniine

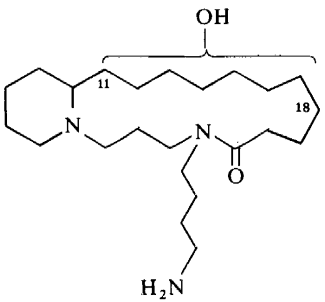
In the meantime there has also been isolated from the plant extract in very small yield, a minor alkaloid, a hydroxy-oncinotine (**18**)[25]. Acetylation gave *N*,*O*-diacetyl-hydroxy-oncinotine with i.r. bands at 1733 cm^{-1} (ester), 1676, 1631 and 1524 cm^{-1} for the amide functions. In the n.m.r. spectrum (100 MHz) of the diacetate there appeared at 5.25 p.p.m. a broad signal (1H) which comes from the grouping



Analogous to oncinotine (**8**) acid hydrolysis of **18**, followed by esterification and acetylation gave the *N*,*N*',*O*-triacetyl methyl ester, **19**. In the mass

Scheme 4.


HO2C—(CH2)8—CO2CH3 +
CH2Li
(a) Ether
(b) MeOH/HCl
CH2—CO—(CH2)8—CO2CH3
(a) Ethane-1,2-dithiol/BF3
(b) Raney-Ni
H2/Cat.
(CH2)10—CO2CH3
16
(a) CH3I
(b) H2/Pt
(±) 12
CH2 = CH-CN
CN
CH3CO·NH—(CH2)4·N—(CH2)3—Br
Ts
H2/Pt/Ac2O
HNCOCH3
(a) H3O⊕ (b) CH3OH/HCl
(c) Br(CH2)3—CN
NH
(a) H3O⊕
(b) MeOH/HCl
(c) Ac2O
(±) 10
HCl
NH2
CO2H
17
3Cl⊖
⊕NH3
(a) SOCl2
(b) Et3N
(±)8



18

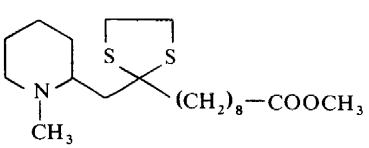
spectrum of **19** there appeared, as with that of **10**, marked peaks at *m/e* 296 and *m/e* 213 (cf. *Scheme 1*). It follows that the *O*-acetyl group is not located in the spermidine or piperidine part of the molecule.

Hydrolysis of **18**, followed by esterification gave the amino-acid ester **20**, which can be oxidized to a ketone with chromium trioxide in dilute aqueous sulphuric acid/acetic acid. The *N,N'*-diacetyl-keto-amino acid methyl ester **21** formed by acetylation shows in the i.r. spectrum the following bands: 1 742 cm^{-1} (ester), 1 712 cm^{-1} (ketone) and 1 675 cm^{-1}, 1 639 cm^{-1} and 1 520 cm^{-1} (amide functions). It can be concluded from the i.r. absorption that the keto group—and the hydroxy group in the alkaloid—cannot be located in either β or α position to an ester function, i.e. at C(19) or C(20) of hydroxy oncinotine. The compound **21** fragments in the mass spectrometer in a manner corresponding to that of the compound **10** (*Scheme 1*). A peak of weaker intensity was registered at *m/e* 436 ($M + - {}^{\cdot}CH_2COOCH_3$) which indicates that in **21** there is no α-keto-ester grouping present[25]. The keto group in **21** which does not induce any specific fragmentation can only be at one of the C-atoms 11–18.

As it is known[26] that dithioethylene ketals, like ethylene ketals[27], can determine the mass spectral fragmentation pattern (α-splitting), the model compound **22** was made and examined.

As the expected fragments (*m/e* 216 and 275) from α-splitting of the dithioketal showed only *ca.* 0.3 per cent of the intensity of *N*-methyl piperideinium *m/e* 98, this method is useless for determining the position of the keto grouping in **21**.

When **21** was submitted to the Schmidt reaction only starting material was isolated. On the other hand when hydroxy oncinotine (**18**) was treated with sodium borohydride in aqueous methanol at room temperature, oncinotine (**8**) was formed by hydrogenolysis of the C—OH-bond. These two experiments



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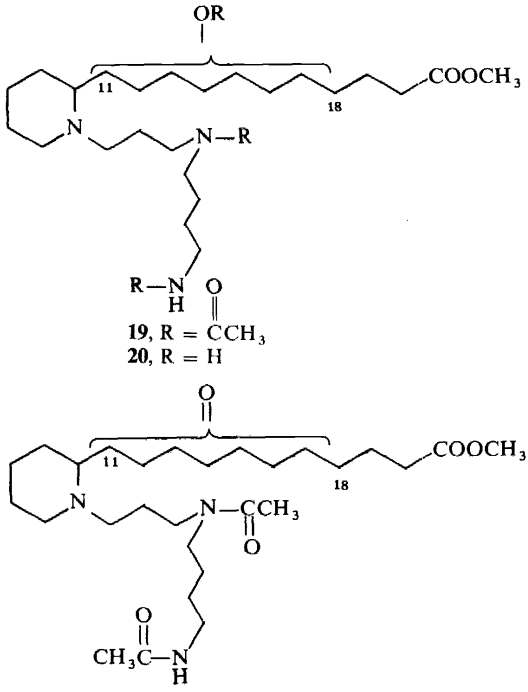


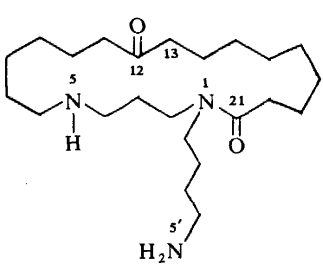
21

point to the functional group, i.e. the hydroxyl bearing C-atom, being influenced through N(5).

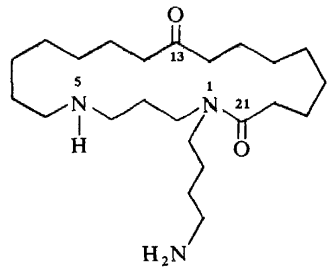
Taken together with the observations that the inandenine alkaloids (see later), isolated from the bark of *O. nitida*, possess —OH and =O groups at C(10) and C(11), a hydroxyl function at position 11 in hydroxy oncinotine is preferred. Scarcity of material prevented further investigations.

2.2. Inandenines

From the leaves of *Oncinotis inandensis* Wood *et* Evans, also indigenous to South and West Africa, a 1 : 1 mixture of two isomeric spermidine alkaloids, each containing a 21-membered lactam ring, was isolated. These two alkaloids, inandenine A (**23**), and inandenine B (**24**), now known respectively



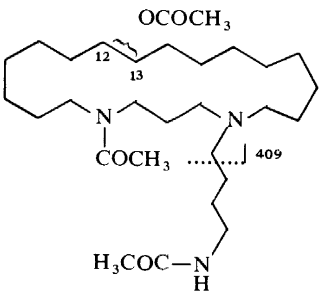
23, Inandenine A
(= Inandenine-12-one)



24, Inandenine B
(= Inandenine-13-one)

as inandenine-12-one and inandenine-13-one*, $C_{23}H_{45}N_3O_2$, differ only in the position of the ketone group[28].

Their slight structural difference has so far prevented separation of the two amorphous bases. The hydrochloride of the mixture, however, is crystalline. Their structure determination is based essentially on the following experiments: The nature of the functional groups was elucidated in a manner similar to that used for oncinotine (**8**). Again the Kuhn–Roth oxidation showed the absence of a (C)—CH_3 group while potassium hydroxide fusion gave spermidine. Reduction with $LiAlH_4$, followed by acetylation gave the inandenine derivative **25**, which in the mass spectrometer loses by α-splitting a fragment corresponding to the putrescine part of spermidine, with formation of the ion *m/e* 409.



25 ($M^+ = 509$ *m/e*)

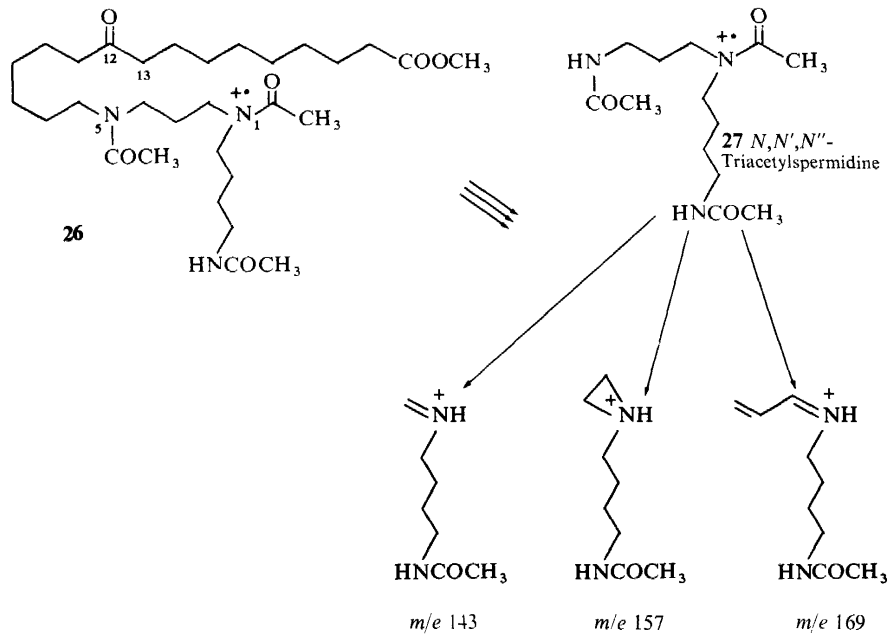
Linking of the C_{16}-chain present in inandenine ketone with the nitrogen atoms 1 and 5 of the spermidine part is to be concluded from the mass spectrometric fragmentation of the ester-acetate **26**, analogous to **10** (*Scheme 5*). This compound fragments essentially as does *N,N',N''*-triacetyl-spermidine (**27**). The main peaks in the spectrum of the latter are *m/e* 143, 157 and 169; they are derived by fission of the 1,3-diamino-propane unit. The mechanism for the formation of these ions has been formulated[16]. The fission of **26**, an example of an inandenine-12-one derivative, takes place in an analogous way.

The fragmentation of **26**, however, is not initiated solely from N(1) but also—in contrast to **27**—from N(5) as charge carrier. This leads to the ions *m/e* 312, 326 and 338 (*Scheme 6*). This is due to N(5) and N(1) containing tertiary amide nitrogens while in **27** only the middle nitrogen is of a tertiary nature.

The mass spectra of the inandenine-ones and of **26** give no information as to the position of the keto group; in any case it cannot, from the given results, be located in the spermidine part. To solve this question, the ester acetate **26** was converted into the mixture of cyclic ethylene ketals **28** and

* In future the term inandenine will be used for alkaloid skeletons which do not contain a ketone group.

Scheme 5.

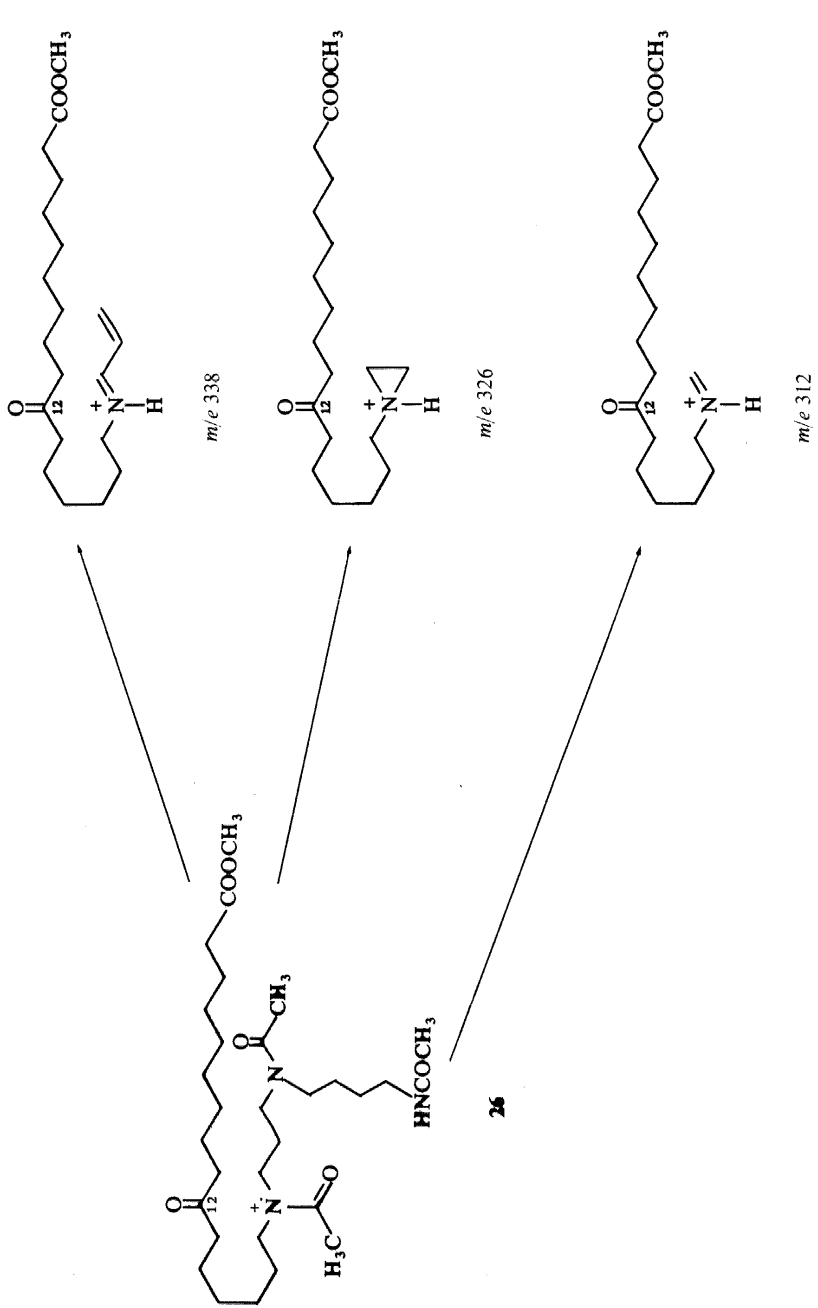


29. The ketal group now takes part in determining the fragmentation pattern (*Scheme 7*)[27]. Besides the fragmentation characteristic for **26** two extra pairs of fragment ions are observed, namely *m/e* 257 + 426 and *m/e* 243 + 440. It follows that in the ketal mixture, the ketal group is located at both C(12) and C(13). This means that in the alkaloids isolated from the leaves of *O. inandensis*, we are dealing with a mixture of two isomers, inandenine-12-one (**23**) and inandenine-13-one (**24**).

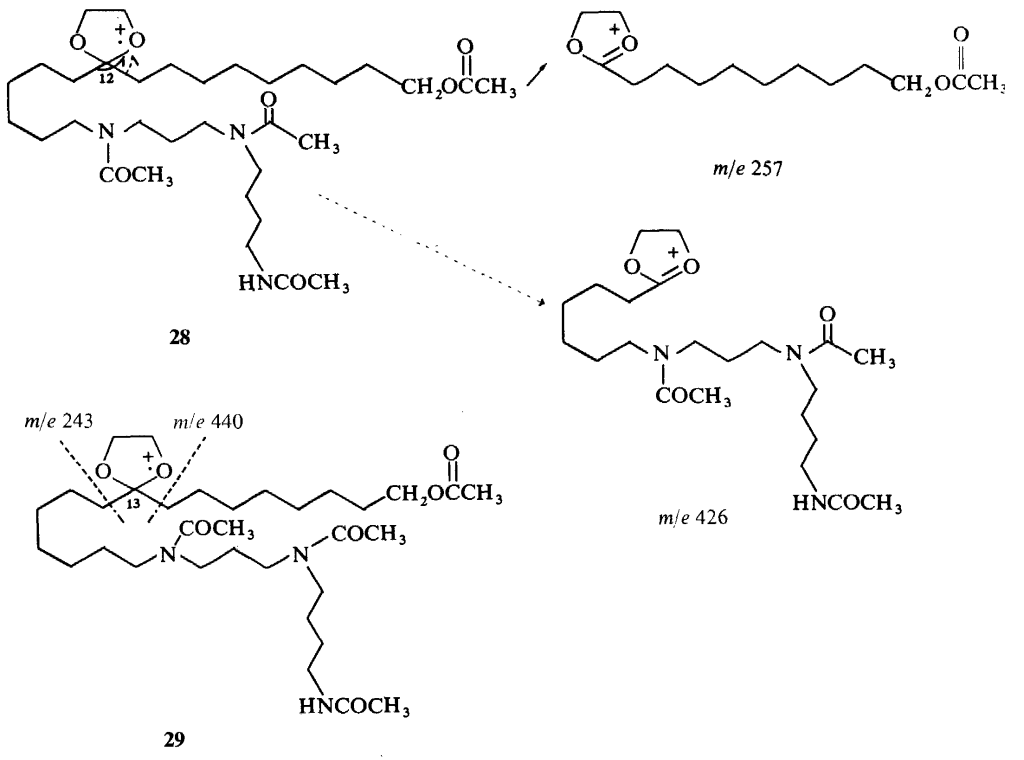
The formulae derived for the inandenine-ones is based mainly on mass spectral analyses. These can be confirmed by chemical degradation. Starting from the inandenine-one mixture the Schmidt rearrangement (using HN_3 in sulphuric acid/chloroform) gives a mixture of four di-lactams **30** to **33**. This mixture was hydrolysed with aqueous hydrochloric acid and the dicarboxylic acids formed separated off and, as their dimethyl esters **34** and **35**, identified by gas chromatography (capillary column). After separating off the dicarboxylic acids, the residue was esterified with methanolic hydrochloric acid, then acetylated and finally the resulting mixture was separated on a silica gel plate. Three spots were obtained which consisted of mixtures of **36** and **37**, **38** and **39** and of the tetramine derivatives **40** and **41** (see *Scheme 8*). Identification was established by mass spectrometry[25].

During the search for further constituents of *O. nitida* definite proof of the presence in the leaves of this plant of inandenine-12-one (**23**) and of inandenine-13-one (**24**) was found; in the bark however, they were not found. Instead, in their place, after acetylation (acetic anhydride/pyridine) inandenine-10-one (**42**) as the *N,N'*-diacetyl derivative **43** and inandenine-10,11-diol (**44**)

Scheme 6.


COOCH3
O
12
+
N
H
m/e 338
COOCH3
O
12
+
N
H
m/e 326
COOCH3
O
12
+
N
H
m/e 312
COOCH3
O
12
+
N
H3C
O
N
CH3
O
HNCOCH3
26

Scheme 7.



as the *N,N',O,O'*-tetraacetate **45** were isolated in small amounts*. Thr separation of **43** and **45** is brought about via the bisulphite addition compound of **43**.

The structure **42** for inandenine-10-one is arrived at as follows: The mass spectrum of the diacetate **43** is practically identical with that of the *N,N'*-diacetyl-inandenine mixture.

With hydroxylamine there is formed a mixture of *syn*- and *anti*-oximes (*syn*-**46** and *anti*-**46**) which, surprisingly, can be separated chromatographically†.

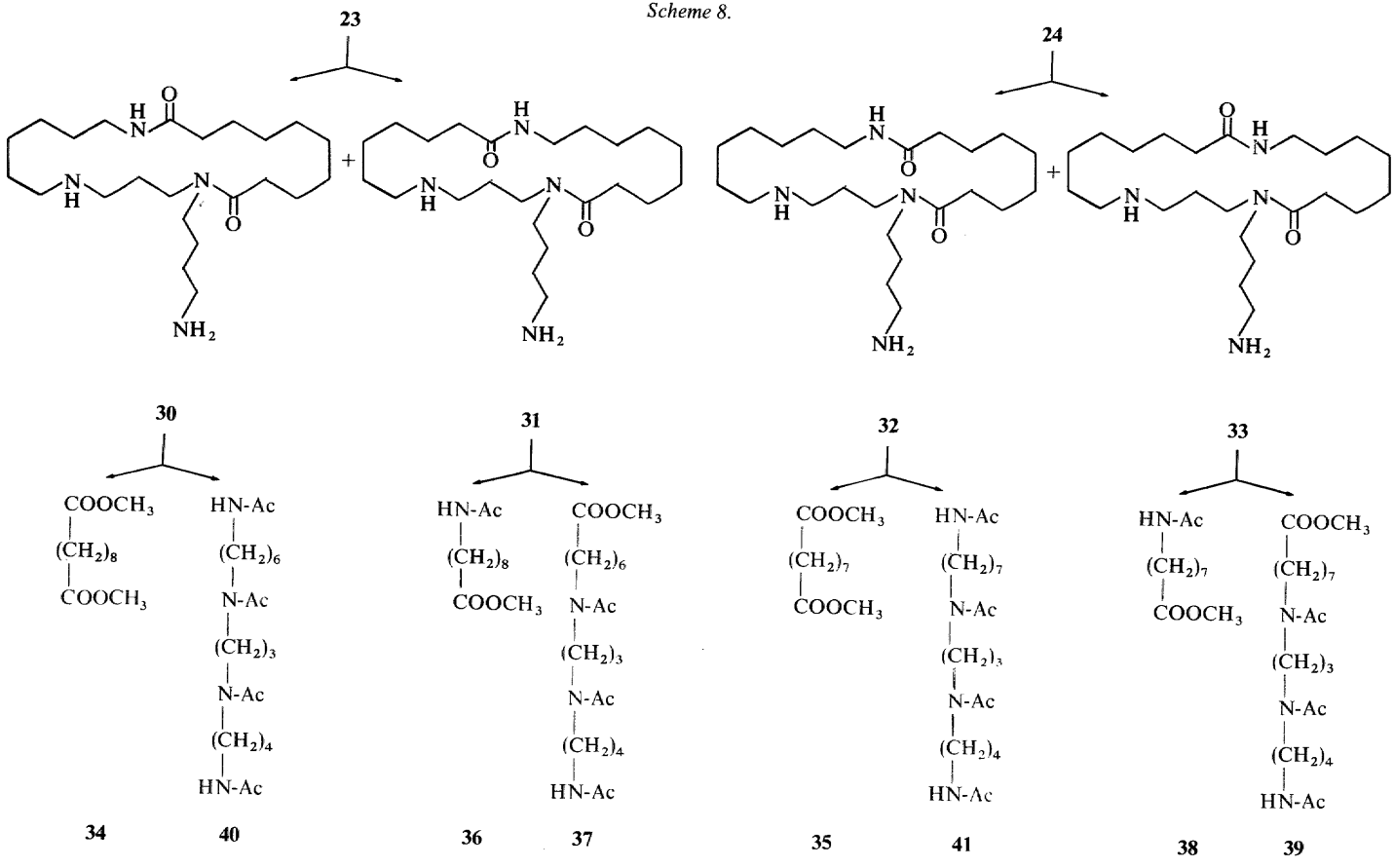
Beckmann rearrangement with sulphuric acid and acid hydrolysis gave decane-1,10-dicarboxylic acid (identified as the methyl ester). The basic products of the hydrolysis were esterified, acetylated and characterized as previously described, see *Scheme 9*.

The constitution of inandenine-10,11-diol (**44**) was arrived at in the following way: its acetate **45** was completely hydrolysed, during which, by a

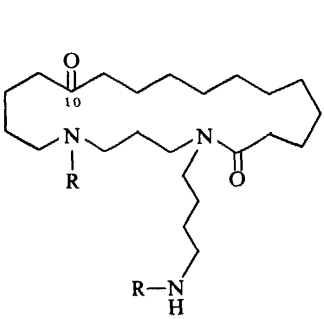
* These acetyl derivatives are not present in the plant.

† The different migration on silica gel is probably due to the formation of an internal hydrogen bonding between the group, =N—O—H, and an amide group in the *syn*-oxime (**46**).

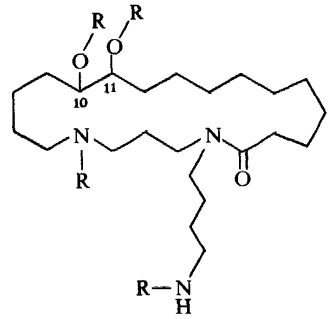
Scheme 8.



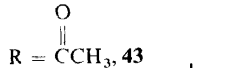
Scheme 9.



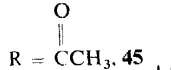
R = H, **42**, Inandenine-10-one



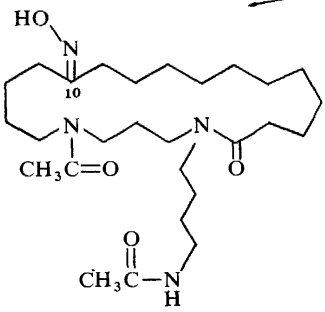
R = H, **44**, Inandenine-10,11-diol



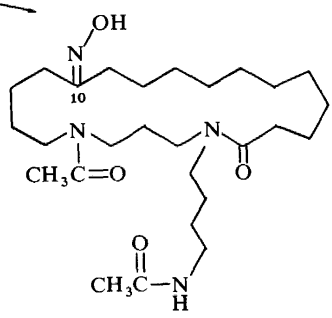
R = CCH_3, **43**



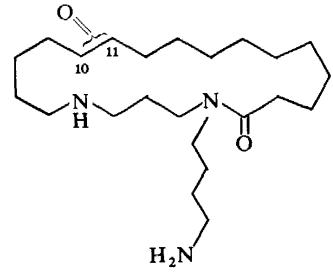
R = CCH_3, **45**

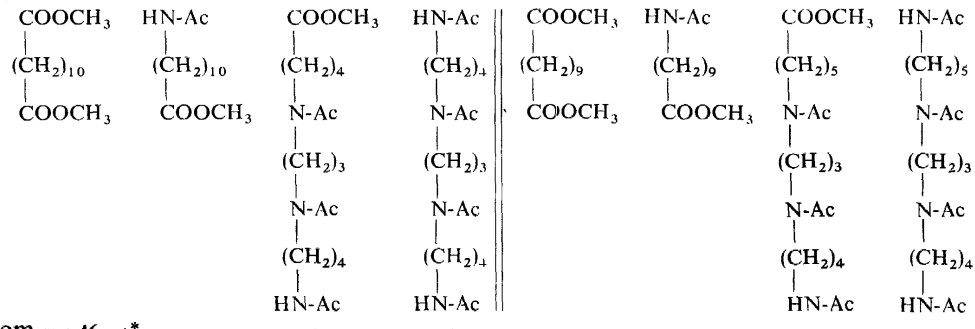


syn-**46**



anti-**46**





from *syn*-**46**	+*	++	++	+			
from *anti*-**46**	++	+	+	++			

* ++ = main product
\+ = side product

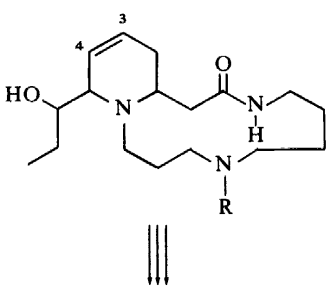
hydride shift, there was formed from the glycol group an inseparable mixture of inandenine-10-one and inandenine-11-one. This mixture—as described for inandenine-12-one and inandenine-13-one—was degraded by the Schmidt reaction. From the degradation products (*Scheme 9*) it can be seen that the inandenine mixture obtained from the alkaloid **44** carried the ketone groups on C-atoms 10 and 11; it follows that **44** is the formula for the inandenine-diol*.

The biogenetic relationship of the inandenines from the bark of *O. nitida* to the oncinotines **8**, **9**, **18** is apparent.

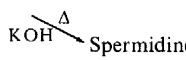
2.3. Palustrine and palustridine

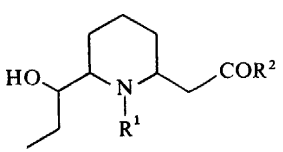
The oncinotines and the inandenines are obviously built up from spermidine and a functionalized palmitic acid unit. The combination of the base with a substituted C_{10}-carboxylic acid is found in the horse-tail alkaloids palustrine (**47**)[20,29] and palustridine (**48**)[29].

Palustrine, $C_{17}H_{31}N_3O_2$, a toxic, major alkaloid of marsh horse-tail ('Duwock', *Equisetum palustre* L.) cannot be isolated easily. As previously mentioned it contains a lactam ring, and yields spermidine[15] on alkali fusion. Catalytic hydrogenation followed by Eschweiler–Clark methylation gives first *N*-methyl-dihydropalustrine. This bis-tertiary base was then reacted with methyl iodide and the quaternary product submitted to Hofmann degradation. The des-base mixture obtained was reduced catalytically and the product boiled for several hours with strong hydrochloric acid.



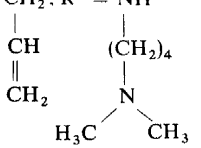
47, Palustrine: R = H
48, Palustridine: R = CHO
49, 3,4-Dihydropalustrine: 3,4-H_2; R = H





50, Dihydropalustraminic acid:
$R^1 = H$; $R^2 = OH$

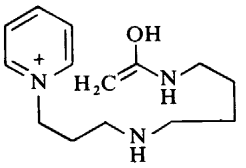
51. $R^1 = CH_2$; $R^2 = NH$



* Whether **44** actually occurs as such in the plant or as the epoxide cannot be stated.

Along with other cleavage products dihydropalustraminic acid (**50**) was isolated and identified by derivatives and spectroscopic data. Dihydropalustrine (**49**) can be broken down further to the des-base **51**, evidence for **49** being the formula for dihydropalustrine. In the mass spectrum of palustrine (**47**) the sidechain is the first fragment to be eliminated, by α-splitting from the molecular ion ($M^+ = 309$), after which, by a McLafferty rearrangement, one of the H-atoms attached to the C(2) atom appears at the lactam oxygen of the fragment ion *m/e* 250. From this follows the position of the double bond in the piperidine ring.

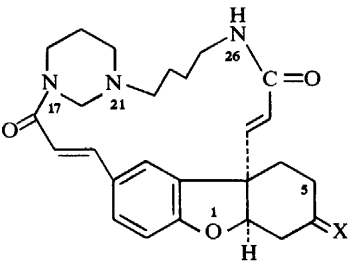
N-formylated palustrine is present in *E. palustre* as the minor alkaloid, palustridine (**48**).



(*m/e* 250)

2.4. Lunaria alkaloids*

The amide-like combination of putrescine (**1**) with *p*-hydroxy-cinnamic acid to give alkaloids is also found in the base, spermidine (**2**). First to be noted are the Lunaria alkaloids, lunarine (**7**, $C_{25}H_{31}N_3O_4$)[12], alkaloid LBY[30], alkaloid LBX (**52**)[30] and LBZ (**53**)[30], lunaridine (**54**) as well as the bases numismine and lunariamine, the structures of which have not yet been established[31]. Formula **7**, derived by x-ray crystallography for lunarine is mentioned in the introduction. Its reduction product, with a secondary alcohol group in place of the keto group, is also known to occur naturally (alkaloid LBY[30]).

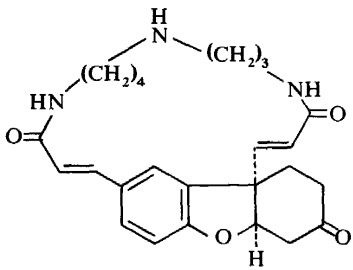


52, Alkaloid LBX: X = =O

53, Alkaloid LBZ: X = 

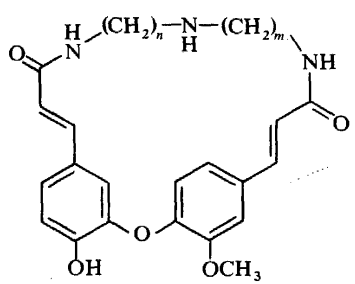
* A detailed discussion of these alkaloids is given by E. W. Warnhoff[32].

The alkaloids LBX and LBZ possess an additional CH_2-group with respect to **7**[30]. Earlier it had been assumed that in these bases the C-atom 5 of lunarine (**7**) and of alkaloid LBY was attached through an additional CH_2-group to the middle N-atom of the spermidine part of the molecule. According to more recent investigations[32]*, however, the CH_2 group is attached to both N-atoms, 17 and 21, as shown in formulae **52** and **53**. LBX is readily formed by treatment of lunarine (**7**) with formaldehyde. Recently, mention has also been made of lunaridine (**54**), which differs from lunarine (**7**) in that the



54, Lunaridine

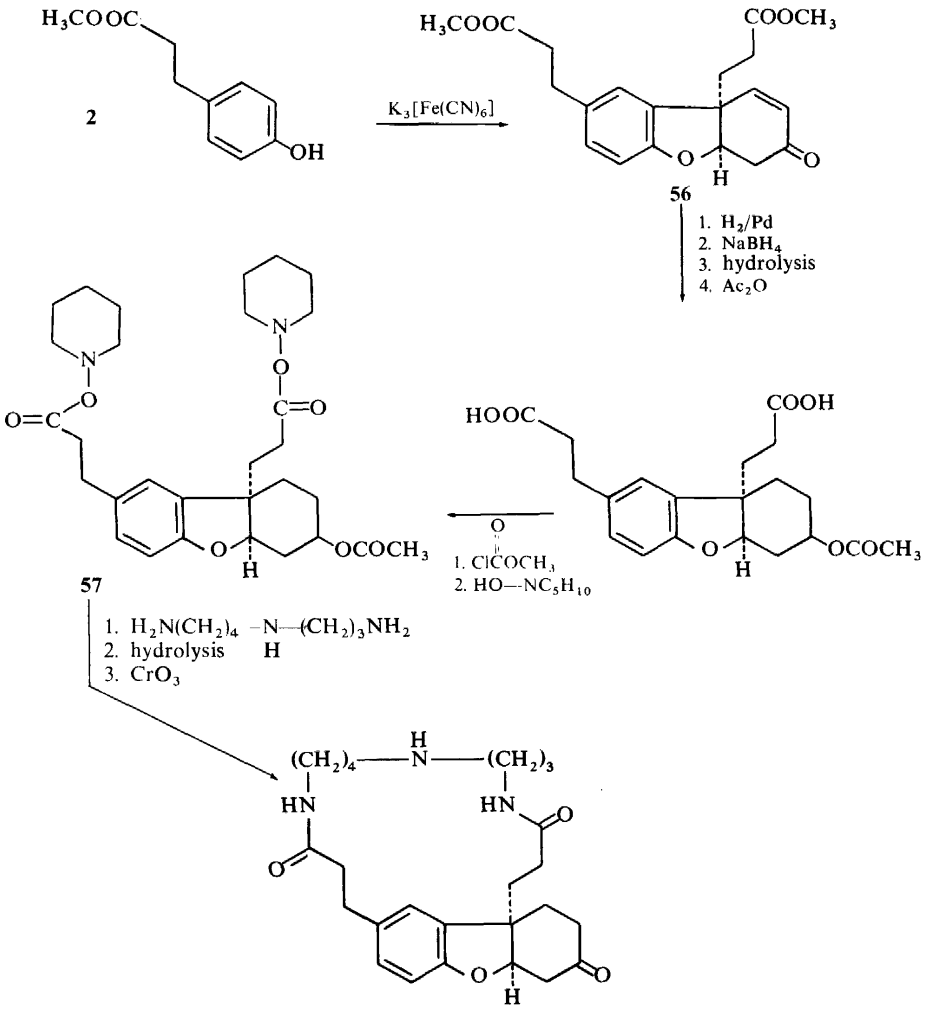
incorporation of the spermidine part takes place in the opposite way. Its tetrahydro derivative (**55**) has been synthesized in the racemic form (cf. *Scheme 10*): By oxidative coupling of *p*-hydroxy cinnamic acid the Pummerer-ketone-like intermediate **56** is formed; this is converted into the *N*,*N'*-diacylhydroxylamine **57** in the manner depicted. Heating the latter with spermidine in boiling tetrahydrofuran gives *ca.* 12 per cent yield of a macrocyclic substance which can be converted into tetrahydrolunaridine (**55**). It is very remarkable that the spermidine is incorporated in a regiospecific manner[33].



58, $n = 3, m = 4$
59, $n = 4, m = 3$
} Codonocarpine

* Private communication from Professor P. Potier, Spring 1971.

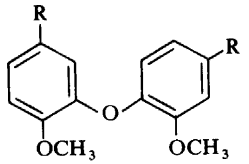
Scheme 10.



55, (±)-Tetrahydro-lunaridine

An alkaloid of the Lunaria class, codonocarpine (**58** or **59**, $C_{26}H_{31}N_3O_5$) has recently been isolated from the bark of *Codonocarpus australis* A. Cunn. (Phytolaccaceae)[34]. This alkaloid, like the spermine alkaloid chaenorrhine to be discussed later, contains a diphenyl ether group.

Acid-catalysed hydrolysis gives spermidine, whereas oxidation followed by methylation affords the diphenylether derivative **60**. By hydrolysis followed by esterification, tetrahydro-codonocarpine can be converted into a compound to which the structure **61** has been assigned. Based on these degradation products codonocarpine has either formula **58** or **59**[34].

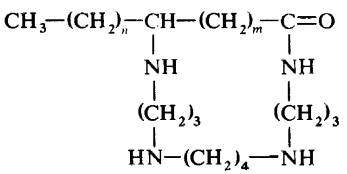


60, R = $COOCH_3$
61, R = CH_2—CH_2—$COOCH_3$

3. SPERMINE ALKALOIDS

3.1. Pithecolobines

Pithecolobine was known to consist of a substituted fatty acid condensed with spermine* and, recently, it has been shown to consist of a series of closely related analogues with the general formula **62**. By a combination of gas chromatography and chemical degradation it was demonstrated that one component (*ca.* 24 per cent) was the compound with structure **63**[35,36], the desoxy derivative of which was synthesized[36]. The main component of the pithecolobine mixture is the compound **64**.



62, Pithecolobines, $m + n = 9,10,11$
63, $m = 3, n = 6$
64, $m = 1, n = 8$ (40–49%)

3.2. Homaline

The alkaloid homaline **65** ($C_{30}H_{42}N_4O_2$) is built up from spermine (**3**) and two cinnamic acid units: it was isolated from *Homalium pronyense* Guillaum. (Homaliaceae)[37]. Recently, from the same plant three new alkaloids structurally related to homaline have been isolated. In one, hoprominol, the phenyl rings of homaline (**65**) are replaced by a β-hydroxyheptyl sidechain and an amyl sidechain. In hopromalinol one phenyl group is replaced by a β-hydroxyheptyl residue. The third alkaloid, hopromine, contains an amyl and a heptyl sidechain instead of the two phenyl groups[37a]. To homaline was finally assigned the formula **65** with the absolute configuration at centres 2 and 2′ as shown[38,39]. The base **66** (the structure of which allows two formulations for homaline[38]) was obtained[37] by Hofmann degradation of this alkaloid.

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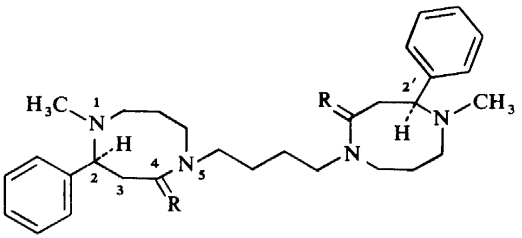
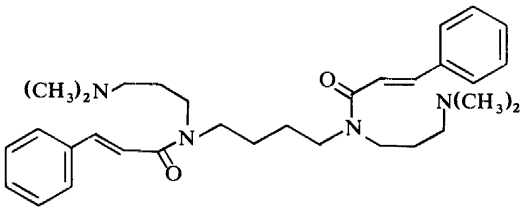
* For earlier work see[17,18].

The correctness of formula **65** follows from the synthesis of the bis-desoxo-derivative **67**, from two moles of (*S*)-*N*-methyl-β-phenyl-β-alanine[39,40] cf.[32].



65, R = O, Homaline
67, R = H_2



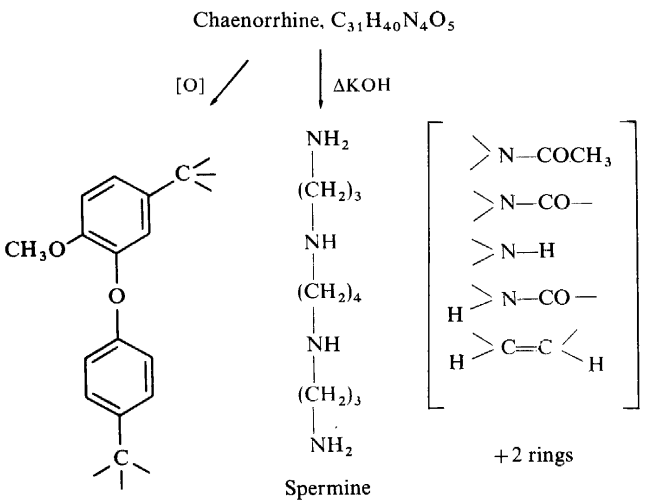
66

3.3. Chaenorrhine

One of the most interesting of the spermine alkaloids is chaenorrhine (**68**) isolated from those parts of *Chaenorrhinum origanifolium* (L.) Wilk. *et* Lge. (Scrophulariaceae) found above ground level[41]. The optically active monobasic alkaloid has an empirical formula, $C_{31}H_{40}N_4O_5$. From spectroscopic data (particularly i.r.), acetylation and reduction, both catalytically and with di-isobutyl aluminium hydride (DIBAH)—($LiAlH_4$ always gave complex mixtures)—the functional groups depicted in *Scheme 11* were arrived at for the alkaloid. Potassium hydroxide fusion gives spermine (**3**). This base is also formed by hydrolysis of tetrahydrochaenorrhine or its *N*,*O*-diacetate **69**. Tetrahydrochaenorrhine can be obtained by reduction of the C,C-double bond and splitting of the diphenyl ether group with sodium in liquid ammonia. The structural features on the left side of *Scheme 11* are deduced from the isolation of 2-methoxy-4',5-dicarboxy-diphenylether from the oxidation of chaenorrhine with potassium permanganate.

The n.m.r. spectrum of chaenorrhine shows, in addition to the signals for the aromatic protons, the *cis*-double bond, the methyl and acetyl groups and others, a multiplet in the region of 3.8–4.0 p.p.m. which, on acetylation, is displaced to 5.7 p.p.m. We have assumed that this proton is that of a benzylamine Ar—$\underline{CH}$—N$\overset{H}{\diagdown}$. The same signal is also found with derivatives of chaenorrhine such as the tetrahydro compound. Only after acetylation to **69** does the latter show a strong signal at *m/e* 161 in the mass spectrometer.

Scheme 11.



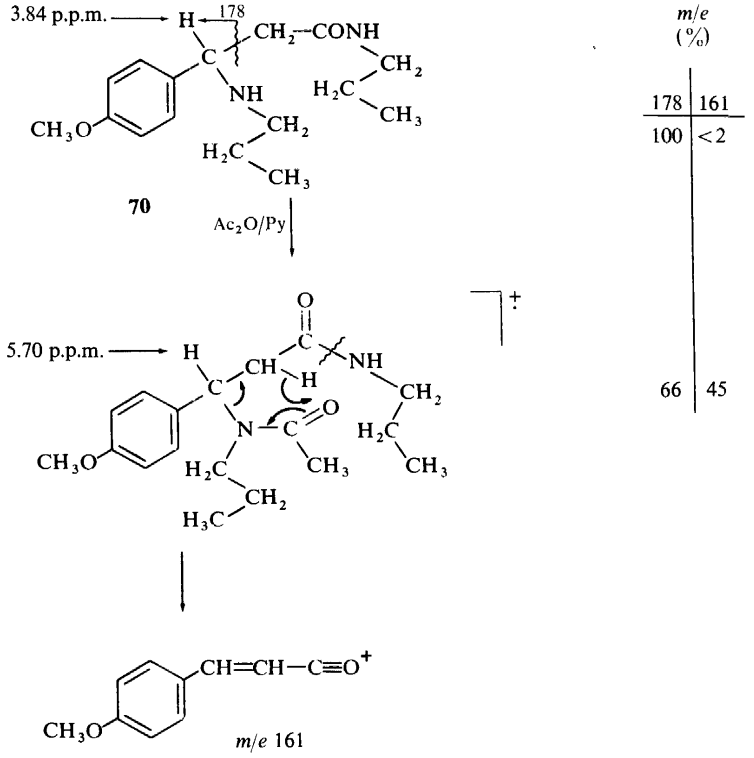
To clear up these relationships the model compound **70**, shown in *Scheme 12*, was investigated. In the n.m.r. spectrum, the benzyl proton signal appears at 3.84 p.p.m. and after acetylation of the benzyl nitrogen at 5.70 p.p.m. In the mass spectrometer the model compound gives practically no *m/e* 161 fragment, but does so after acetylation by a McLafferty-like rearrangement. From these findings it can be concluded that in *N,O*-diacetyl-tetrahydro-chaenorrhine (**69**) a dihydro-β-amino-*p*-methoxycinnamic acid is present in the structure. This means that one of the primary amino groups of spermine is bound to the diphenylether structural unit at the C-atom in the para-position, as depicted in *Scheme 11*.

Where is the second primary amino group of spermine attached? This question is answered by the experiments brought together in *Scheme 13*. Chaenorrhine possesses four nitrogen-containing functional groups A, B, C, D, as shown, and gives, on reductive fission of the diphenylether group followed by acetylation, the already mentioned *m/e* 161 peak. Treatment of chaenorrhine with DIBAH yields bisdesoxochaenorrhine, which, like the parent compound, only forms a mono-acetate. The two tertiary amide groups A and C are next reduced by treatment with Na/liquid NH_3; this, followed by acetylation, forms a substance which in the mass spectrometer again yields a strong *m/e* 161-fragment. It follows that the above-mentioned dihydro-β-amino-*p*-methoxy-cinnamic acid part is bound to the second primary amino group of spermine. To confirm this tris-desoxo-chaenorrhine was also investigated. The compound gives a *N,N'*-diacetyl derivative and after reduction and acetylation no longer gives the *m/e* 161-fragment.

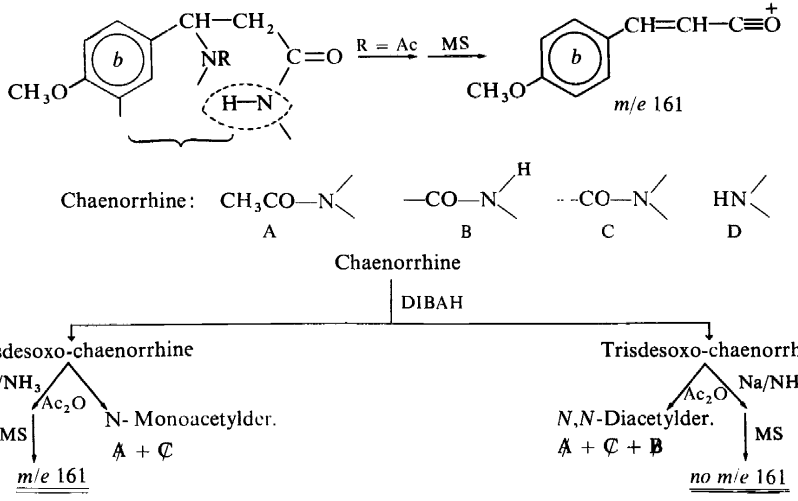
In *Scheme 14* the partial structure of *N,O*-diacetyl-tetrahydro-chaenorrhine is given. The compound gives in the mass spectrum in addition to the fragment *m/e* 161, the fragment *m/e* 107 of the second phenyl nucleus. Acid hydrolysis of this compound yields, in addition to spermine (**3**), *p*-hydroxy

Scheme 12.

Fragmentation of a model substance

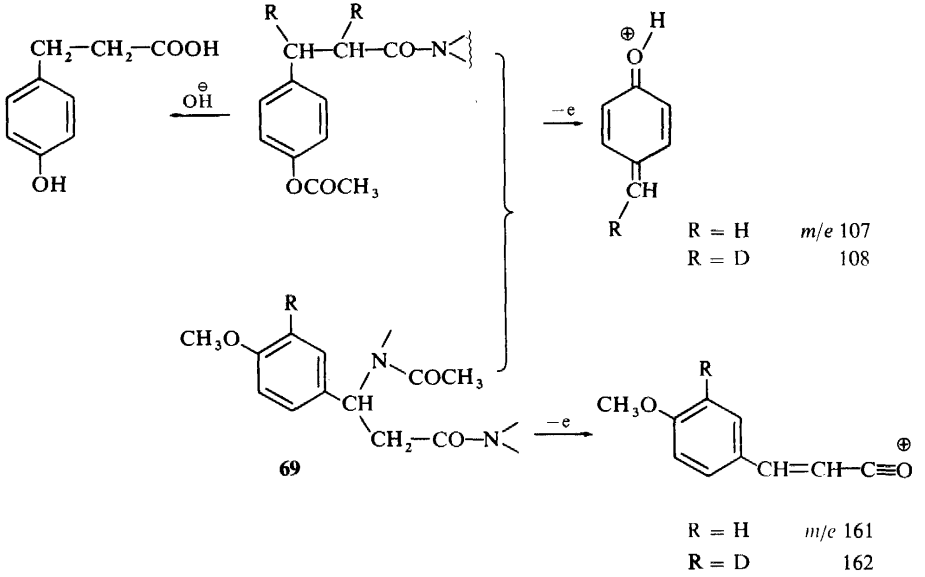


Scheme 13.

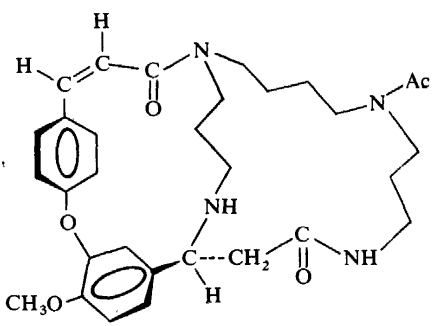


Scheme 14.

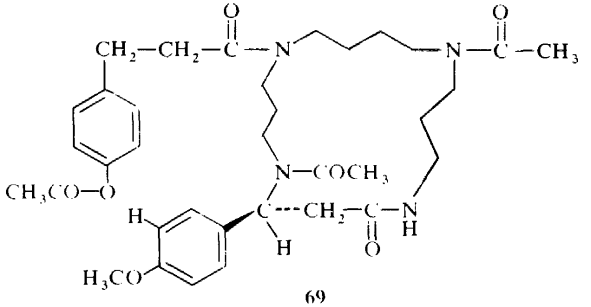
Partial structure of N,O-diacetyltetrahydrochaenorrhine



dihydrocinnamic acid. These relationships were verified by preparing the trideuterated reduction product (R = D). As expected the fragment ions were shifted from *m/e* 161 to *m/e* 162 and *m/e* 107 to *m/e* 108. From what has been said, a chaenorrhine formula, in which the two primary amino groups of spermine (**3**) are joined to the *p*-methoxy-cinnamic acid unit, can be derived. From this it follows that for chaenorrhine there are two possible formulae, in which the ring containing the diphenylether grouping is either seventeen- or nineteen-membered. As can be seen from *Figure 1* the u.v. spectrum of chaenorrhine differs widely from that of the diphenylether model; in our opinion this is due to the two phenyl rings of the diphenylether grouping not being in the same plane. Working with space-filling models it can be shown

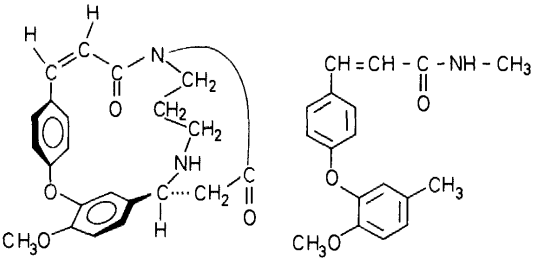


68, Chaenorrhine



69

that an approximately planar conformation of the diphenylether grouping is possible when there is a nineteen-membered ring present, thus giving for chaenorrhine the formula **68** with its seventeen-membered ring. (Further investigations are in progress.) This formula **68** would lead one to expect that in c.d., owing to the inherent disymmetric chromophore, strong Cotton effects would appear; these should disappear on breaking the diphenylether bridge.



1 Chaenorrhine 2

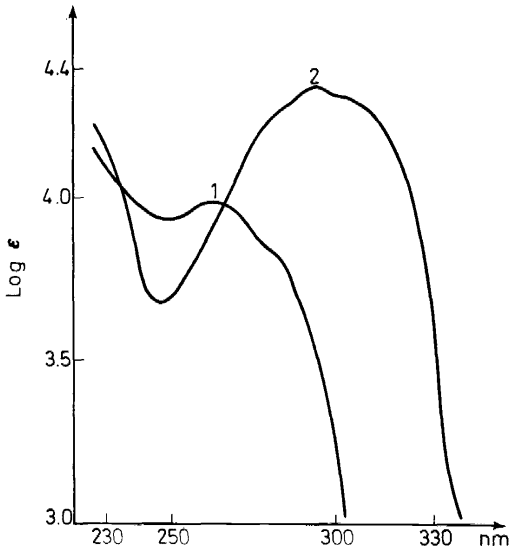


Figure 1

In fact with both chaenorrhine (**68**) and its dihydro derivative, strong Cotton effects are found which are practically absent in the tetrahydro compound.

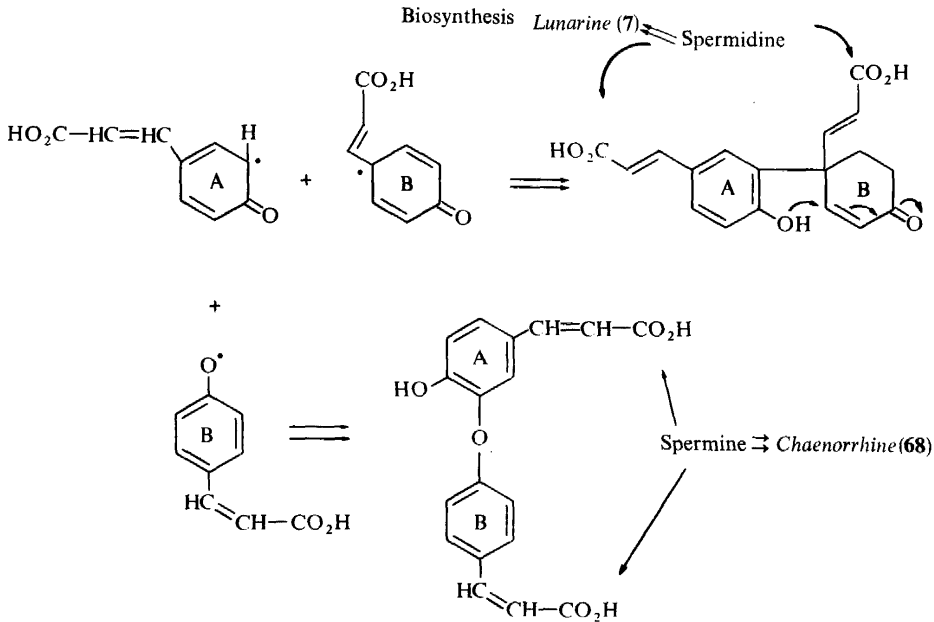
The absolute configuration of the benzylic chiral centre was obtained by correlation of the c.d. curve of tetrahydrochaenorrhine with that of *R*-(+)-ethyl-1-(4-methoxyphenyl)-ethylamine[41].

4. OBSERVATIONS ON BIOSYNTHESES

As indicated earlier, the biosyntheses of the macrocyclic spermine and spermidine alkaloids can be pictured as the joining of the two bases spermine (**2**) or spermidine (**3**) with substituted fatty acids. It is interesting to note that 10,16-dihydroxy palmitic acid appears as a building stone for plant cuticle. A palmitic acid with functional groups at these C-atoms could form the basis of inandenine-12-one[42].

It is further remarkable that in the 'aromatic' alkaloids, the cinnamic acid building stone always appears. A possible biogenetic path for lunarine (**7**) and chaenorrhine (**68**) is shown in *Scheme 15*. The 'biogenetic-like' pathway

Scheme 15.



was elegantly used in the synthesis of (±)-tetrahydrolunaridine (**54**) (*Scheme 10*)[33].

The macrocyclic spermine and spermidine alkaloids found to date do not appear to be associated with any particular plant family. It is to be expected that further examples of these interesting, but often in no way readily available and easy to isolate, plant bases will be discovered.

For support of the work carried out in the Zürich laboratories, we are much indebted to the 'Schweizerischer Nationalfonds'.

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